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Four easy pieces: mechanisms underlying circadian regulation of growth and development

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The circadian clock confers rhythms of approximately 24 hours to biological events. It elevates plant fitness by allowing plants to anticipate predictable environmental changes and organize life process to coincide with the most favorable environmental conditions. Many developmental events are circadian regulated to ensure that growth occurs at the ideal time or season relative to available resources. Circadian clock control over growth and development is often achieved through regulation of key phytohormone action. Circadian influence over the genome is widespread and the clock modulates genes involved in phytohormone synthesis and signaling, in addition to other pathways shaping growth and development. This review presents four nonmutually exclusive mechanisms by which temporal information is gleaned from the core oscillator and passed to pathways regulating plant growth and development.

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Introduction

The circadian clock is an endogenous timekeeper that times life processes to specific parts of the day and allows organisms to predict environmental cycles, such as day/night and seasons, that result from rotation of the Earth. Anticipatory behavior provided by circadian clock is profoundly important to plants, as plants are sessile and are heavily dependent on their immediate environment for survival. Circadian rhythms increase the efficiency of physiological processes by synchronizing the timing of these processes with both optimal environmental conditions and other related activities. Fitness and adaptation are markedly increased when endogenous circadian timing matches environmental cycles [1]. Circadian influence over plant development spans the entire life cycle, including early events like seed germination and later developmental programs such as the floral transition [2•,3,4]. Plants also possess the capacity for substantial phenotypic plasticity in daily growth, and drastic changes in environmental conditions trigger alternate growth programs, such as shade avoidance syndrome (SAS) and high temperature responses [5,6•,7]. The clock enables these growth programs and acts as a 'fact-checker' that allows plants to distinguish between normal environmental fluctuations and more extreme long-term changes in the environment.

Circadian clock mechanics

Intense research over the past 15 years has revealed many molecular details of the plant circadian oscillator. Here we provide a limited overview of the clock mechanism, as several excellent reviews on this subject have been written recently [8,9]. Like all eukaryotic circadian clocks, the plant oscillator consists of a series of interlocking feedback loops. To date, three negative feedback loops have been elucidated in the Arabidopsis oscillator [10,11]. Morning expression of partially redundant myb-related transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) serves to activate expression of two *PSEUDORESPONSE REGULATOR* (*PRR*) genes, *PRR7* and *PRR9* [12]. This so-called morning loop is closed when PRR7 and PRR9 feedback to inhibit *CCA1* and *LHY* expression [13]. CCA1 and LHY also repress *TIMING OF CAB EXPRESSION 1* (*TOC1*) expression [14], while TOC1 is a key transcription factor within a feedback loop that activates *CCA1* and *LHY* transcription to reinitiate the 24-hour cycle [15]. An additional feedback loop is active in the evening, where GIGANTEA (GI) is a component of an activity that reinforces *TOC1* expression [10]. Like *TOC1*, *GI* expression is repressed by the action of CCA1 and LHY in the early part of the day [16]. The rhythms generated by the action of these proteins are communicated to the manifold areas of plant physiology subject to clock regulation.

Clock influence over phytohormone action

Diurnal rhythms in transcriptional activity are so widespread in Arabidopsis that almost the entire transcriptome has the potential for daily changes in expression. Up to 90% of Arabidopsis genes have cyclic expression in response to combined light/dark and warm/cool cycles, while between 30 and 50% of the transcriptome is rhythmic in a single environmental condition [17•,18•]. The circadian oscillator influences growth and development by ensuring that specific aspects of phytohormone action

occur at defined times of the day, often by imparting rhythmic expression to genes involved in phytohormone conjugation, signaling, and degradation.

A critical developmental 'decision' for plants is seed germination, as this act determines the season in which seedling development begins and also specifies the physical location of the plant for the remainder of its existence. Environmental cues coupled with the circadian clock promote germination by regulating the balance between the stimulatory influence of gibberellic acid (GA) and the inhibitory action of abscisic acid (ABA) [19]. In dry seeds, the circadian clock is arrested in an evening-like state distinguished by high expression of the evening-phased genes *TOC1* and *GI*, along with low transcript levels for the morning genes *LHY* and *CCA1* [2•]. Wetting of Arabidopsis seeds, or imbibition, starts and sets the phase of the transcriptional oscillator [20]; therefore, the circadian clock is running before and at the time of germination. Though imbibition initiates clock behavior, rhythms become more robust and synchronized upon exposure to light or temperature cues [21]. Transcriptional regulation of GA and ABA metabolism genes alters phytohormone levels in germinating seeds [19]. Seeds undergoing germination have increased GA levels relative to dry seeds and an up-regulation in expression of *GIBBERELLIC ACID 3-OXIDASE* (*GA3OX1*). GA3OX1 is the enzyme responsible for the last committed step in the generation of bioactive GAs. Expression of transcripts encoding ABA catabolic enzymes, including the cytochrome P450 *CYP707A2*, increases in germinating seeds along with the rise of GA levels.

Analysis of gene expression and seed germination in Arabidopsis clock mutants reveals that normal phytohormone-related gene expression programs in seeds require proper clock function [2•]. In this capacity, the plant circadian clock likely acts as an integrator of light and temperature signals to ultimately control the action of phytohormones governing germination in seeds. *GA3OX1* levels are elevated in imbibed *lhy cca1* double mutant seeds and low in the *gi* mutant, which likely underlies the enhanced germination efficiency of *lhy cca1* seeds and the poor germination of *gi* seeds. *CYTOCHROME P450 707A2* transcript levels are lower in *gi* seeds, so poor germination may also be due to higher ABA levels in this mutant. Mutations in the core circadian clock genes *LUX* and *ZTL* also reduce germination, demonstrating an intact circadian clock is crucial for optimal germination. Interestingly, circadian expressed ABA-dependent genes and GA-dependent genes exhibit nearly oppositely phased circadian expression in seedlings [17•], suggesting these phytohormones also have opposing roles in developmental processes in more mature plants.

The clock also influences the developmental effects of auxin, largely through regulation of auxin signaling pathways. The effects of auxin are temporally controlled, or gated, by the circadian clock. Gating refers to the circadian oscillator rendering physiological processes active within a specific temporal window, while other cues affect the extent or magnitude of these processes at the permissive time [5]. Circadian regulation of auxin-driven transcriptional responses is a central mechanism for gating of auxin signaling to achieve precisely timed growth. Astoundingly, 56% of auxin-responsive genes are circadian regulated, and the majority of these rhythmic genes have peak expression during the day [22••]. Similarly, genes encoding the canonical positive drivers of auxin signaling, the AUXIN RESPONSE FACTOR (ARF) transcription factors, are day-phased. On the other hand, AUX/IAA genes, encoding negative regulators of auxin-responsive genes, have greatest circadian expression at night. The anti-phase relationship between these two categories of auxin regulatory genes is consistent with the clock gating auxin-dependent transcriptional responses. In agreement, rhythmic hypocotyl growth is enhanced when seedlings are treated with auxin during subjective night under circadian conditions [22••]. All aspects of auxin metabolism and response may collectively reinforce one another to accomplish gated plant growth. Levels of both free and conjugated auxin fluctuate in a circadian fashion, as do many genes involved in auxin biosynthesis, conjugation, and transport [22••,23].

Circadian clock regulation of phytohormone action is likely a common mechanism underlying the pervasive effects of the clock on plant development. In addition to auxin-related genes, large-scale circadian regulation has recently been described for genes contributing to the synthesis of and signaling for GA, ABA, and brassinosteroids (BRs) [22••,24,25,26•]. Our greater appreciation of the extensive influence of the clock in phytohormone pathways, along with the established role of phytohormones in growth and developmental programs, indicates that most, if not all, aspects of plant development are influenced by the circadian system. This review encompasses not so much a list of clock-influenced developmental programs but, instead, focuses on characterized and emerging molecular mechanisms by which temporal information is passed from the core molecular oscillator to output processes (Figure 1). The mechanisms are: firstly, direct binding of core clock proteins to regulatory regions of output genes; secondly, rhythmic chromatin modification; thirdly, physical interaction between core clock proteins and proteins of other pathways; fourthly, rhythmic intermediate transcription factors. Plant cells use these four mechanisms to directly limit or promote activity of pathways in growth and development.

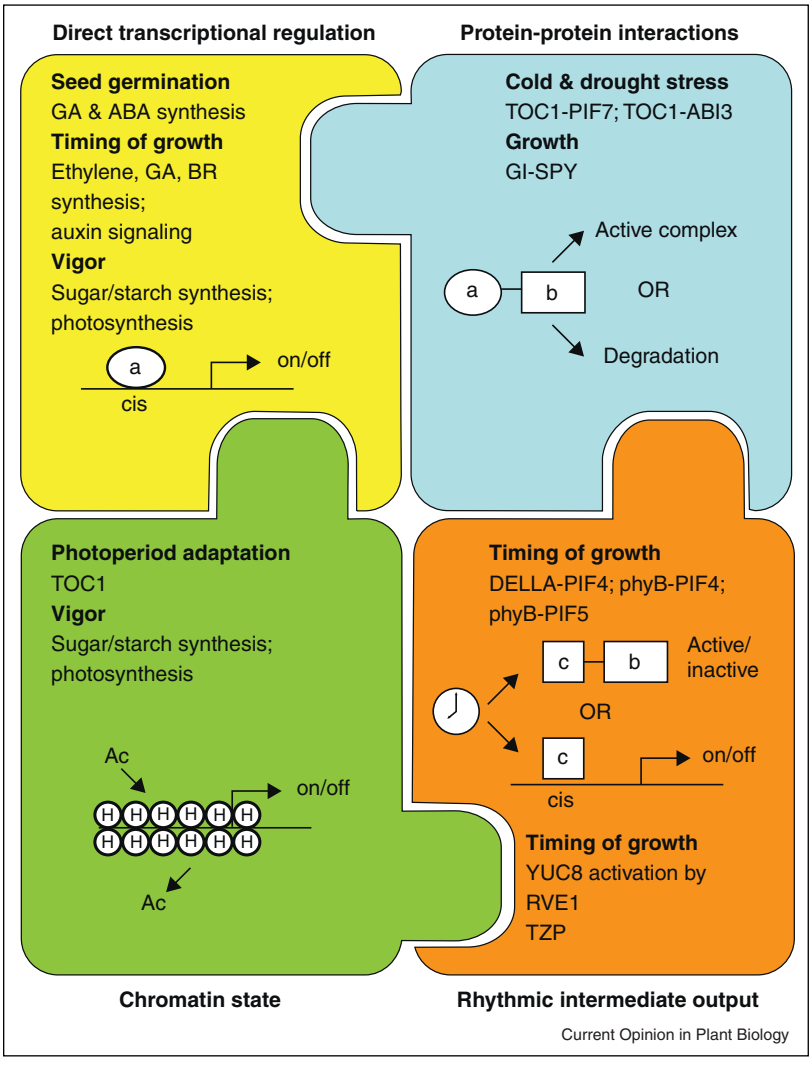
Direct binding of core clock proteins to regulatory regions of output genes

Temporal information is communicated from the oscillator to output genes through direct interaction between

core clock transcription factors and regulatory regions in the promoters of growth and development genes. For example, CCA1 reaches maximal levels in the morning and directly represses evening-expressed genes that include growth-related genes like photosynthesis components and enzymes for sugar and starch synthesis [14,27••,28]. Recent work in Arabidopsis suggests hybrid vigor in F1 hybrids stems, in part, from modification of CCA1-mediated control of these growth genes [29••]. An altered circadian expression pattern for *CCA1* in hybrids leads to extended expression of growth-promoting genes into the evening, a time when CCA1 normally represses expression. These findings underscore the intimate relationship between the core circadian clock and the signaling networks controlling plant growth, as well as highlight how tightly plant growth is regulated with respect to time of day.

CCA1 binds to the evening element (EE) and to the CCA1 binding site (CBS) in the regulatory region of genes to confer rhythmic expression patterns [22••,27••,30,31]. The EE is a nine-nucleotide sequence over-represented in evening-expressed genes [32]. The CBS is also nine nucleotides but differs from the EE in two residues and confers early day expression [33]. Sequences that flank the EE in some promoters are not required for rhythmic expression, but their presence shifts the phase of peak expression to earlier in the day [34]. The core clock protein LHY and at least one member of the clock-related REVEILLE (RVE) family also bind to the EE, and

Figure 1



Four general mechanisms for the transmission of temporal information from the circadian oscillator to developmental and growth programs. Each puzzle piece represents a distinct mechanism as indicated. Within each piece are specific examples that illustrate the fundamentals of the mechanism. Oval labeled 'a' represents a core circadian clock protein, rectangle labeled 'b' represents a nonrhythmic regulatory protein acting within a signaling pathway, and square labeled 'c' represents an intermediate circadian regulated transcription factor. In the green puzzle piece, small circles with 'H' represent histone H3 bound to a promoter region and 'Ac' indicates histone acetylation and deacetylation.

possibly to the CBS as well [35]. Other regulatory sequences are associated with rhythmic gene expression patterns phased to dawn, early day, late day, and midnight [17•,18•,26•]. For example, the HUD element is over-represented in promoters of rhythmically expressed genes that also respond to auxin and BR. Genes with HUD-containing promoters are dawn-expressed under diurnal conditions, a time coincident with maximal hypocotyl growth [18•,26•]. The HUD element is also the focus of clock-regulated light signaling, where the red light photoreceptor phytochrome B (phyB) appears to have a negative role. Diversity in rhythmic gene output may be accomplished by coupling different *cis* elements in the same promoter, the end result being gene expression matched to growth and physiological requirements across a full 24-hour day.

The core clock protein TOC1 binds to the promoter of the ABA-related gene encoding the H subunit of the magnesium-protoporphyrin IX chelatase (ABAR/CHLH/GUN5), thus connecting the circadian oscillator to ABA signaling and related growth processes [36•]. ABAR is proposed to act as an ABA receptor [37,38], and is a critical component of growth under drought and cold conditions. Importantly, plants with altered TOC1 function have ABA-related drought phenotypes, including reduced stomatal aperture and enhanced survival under dehydration stress for the *toc1* mutant. TOC1 also represses *ABAR* expression and phases its waveform to dawn. ABA induces *TOC1* in a clock-gated manner during the subjective day, and this induction requires ABAR. Therefore, TOC1 and ABAR appear to reciprocally regulate each other by forming a feedback loop that accomplishes fine-tuning of circadian responses to ABA.

Rhythmic chromatin modification

The recognition of histone modification as a regulatory mechanism in both plant and mammalian circadian systems demonstrates this is a fundamental means by which the clock exerts control over development [27••,39,40]. In the plant core circadian oscillator, rhythmic induction of TOC1 expression is preceded by acetylation of the N-terminal tails of histone H3 incorporated into nucleosomes associated with the *TOC1* promoter at the EE [27••]. H3 acetylation correlates with binding of the chromatin remodeling factor SSRP1, a component of the facilitates chromatin transcription (FACT) complex, whose association with promoters parallels active transcription [41]. The *TOC1* promoter contains EEs to which CCA1 binds in a rhythmic fashion, with maximum occupancy at dawn [27••]. Association of acetylated H3 at the *TOC1* promoter is increased in the *cca1 lhy* double mutant and decreased in plants constitutively expressing CCA1; therefore, CCA1 is proposed to be involved in recruiting the chromatin modification machinery that antagonizes acetylation of histone H3 at the *TOC1* promoter. The dynamics of chromatin modification events affect rhythmic *TOC1* expression and ultimately influence developmental events like hypocotyl elongation and flowering time [42]. The EE is found in promoters of many output genes; thus, the potential exists for chromatin modification to be a widespread mechanism to promote or repress expression of genes needed for key aspects of growth and development. Rhythmic chromatin modification is likely to have a broader role in the plant circadian clock than is currently appreciated, as circadian changes in chromatin are widespread in the mammalian circadian system [40]. An intriguing possibility is that gating of growth and development may be accomplished by global circadian clock regulation of a permissive chromatin state.

Physical interaction between core clock proteins and proteins of other pathways

Direct interaction between a core clock protein and non-clock proteins influences key growth events. TOC1 abundance peaks in the evening, and any protein–protein interactions it makes at this time have the potential to transmit temporal information to that molecular partner. TOC1 interacts with several proteins that are external to the core oscillator, including basic helix-loop-helix (bHLH) transcription factors of the PHYTOCHROME INTERACTNG FACTOR (PIF) family, which have established roles in cold and drought tolerance, light signaling, and SAS [5,43]. The interaction between TOC1 and PIF7 temporally influences expression of C-REPEAT/DEHYDRATION RESPONSIVE ELEMENT-BINDING FACTOR (*CBF2/DREB1C*) [43]. CBF2/DREB1C is a member of the CBF/DREB1 transcription factor family, which serves to activate hundreds of genes involved in low temperature and drought responsive transcriptional programs. Expression of the *CBF/DREB1* genes is circadian regulated so that peak expression occurs in the morning, presumably to allow their downstream protective gene products to accumulate before night when lower temperatures have the potential to induce mild temperature stress. PIF7 represses CBF2/*DREB1C* by specifically binding to a G-box-containing region in the promoter. PIF7 activity is, in part, regulated by interaction with TOC1. The TOC1–PIF7 complex enhances PIF7 repressive activity and, therefore, limits expression of *CBF2/DREB1C*. Appropriate control of these pathways in unstressed conditions is critical, as hyper-activation of the stress response could divert resources away from normal growth and development.

In addition to PIF7, TOC1 interacts with PIF3 and PIF3-LIKE 1 (PIL1), which are both regulators of phyB-dependent red light signaling, to affect chloroplast development in the case of PIF3 and the end-of-day SAS response in the case of PIL1 [5,44]. TOC1 also interacts with ABI3, a protein with roles in ABA signaling, seed germination, and desiccation tolerance [45]. Additional interactions between clock proteins and nonclock regulators have been identified, such as GI and the GA signaling component SPINDLY (SPY) and GI with the

E3 ubiquitin ligase COP1. The SPY-GI and COP1-GI complexes both contribute to important aspects of phytohormone and light signaling behind hypocotyl elongation and the vegetative to floral transition [46,47].

Rhythmic intermediate transcription factors

Rhythmically expressed transcription factors act as intermediates to convey temporal information to growth and developmental processes. At least 247 Arabidopsis transcription factor genes are circadian regulated, though the processes these proteins control are largely unknown [17•]. The morning-expressed *TZP* (*LIGHT5*) gene encodes a nuclear protein that, in conjunction with blue light, regulates genes required for growth [48]. Some of these target genes, like those encoding peroxidases, are involved in cell wall synthesis and modification, while others are auxin-related genes that act in hypocotyl growth, such as *AXR5*, *DFL1*, *WES1* [48]. The rhythmically expressed myb-like transcription factor *RVE1* exemplifies another clock connection to growth. RVE1 belongs to the same family as CCA1 and LHY and, though not part of the core clock, this transcription factor binds the EE in the auxin biosynthetic gene *YUCCA8* to regulate auxin synthesis in a tissue specific manner [35].

In diurnal conditions, light and the circadian clock together ensure maximum hypocotyl growth at dawn by controlling the presence of PIF4 and PIF5 [49••]. PIF4 and PIF5 are key regulators of a gene network that controls photomorphogenesis, hypocotyl elongation, and SAS [49••,50,51]. *PIF4* and *PIF5* expression is clock-controlled so that PIF4 and PIF5 protein abundance peaks at dawn. Consequently, the rate of hypocotyl elongation is greatest at dawn just before light is present. The onset of light signals at dawn halts growth by inducing phyB-directed degradation of PIF4 and PIF5 via an unknown mechanism requiring the ubiquitin 26S proteasome system [51,52]. Arrhythmic circadian clock mutants that constitutively express *PIF4* and *PIF5* display unrestricted growth from dusk to dawn [49••]. The PIF4/PIF5 regulatory pathway is a paradigm for external coincidence, as the endogenous circadian clock determines the timing of PIF4 and PIF5 expression and the activity of these proteins is sensitive to exogenous light signals.

Regulation of PIF4 activity is also a paradigm for internal coincidence of signals that modify growth in response to endogenous and environmental signals. Seedling hypocotyl elongation is antagonistically regulated by light and GAs. PIF4 activity is inhibited by DELLA proteins, which are transcriptional repressors of GA-dependent growth and development [53••]. DELLA proteins promote growth restraint by interacting with the DNA-binding domains of PIF4 and PIF3, thereby preventing the PIFs from activating transcription of growth-promoting genes. GA triggers 26S proteasome-dependent degradation of DELLA proteins by promoting their interaction with the SCF^{SLY} E3 ubiquitin ligase. Therefore, elevated GA levels release PIF4 from DELLA protein inhibition and subsequently PIF4 is free to stimulate hypocotyl growth. A similar mechanism likely contributes to the dramatic shift in plant morphology associated with exposure to high ambient temperatures, as *PIF4* is induced and required for the elongated hypocotyl, petioles, and leaves typical of Arabidopsis growth at elevated temperatures [6•,7].

Future questions

Little is known of where and how the circadian clock influences production of alternate transcript splice forms and noncoding RNAs (ncRNAs), as well as the consequences of this for growth and development. The extensive pool of alternative splice forms and ncRNAs in the Arabidopsis genome represents a potentially important realm of circadian clock influence. More than 40% of Arabidopsis intron-containing genes exhibit transcripts with alternative splice forms [54]. The Arabidopsis circadian clock regulates alternative transcript splice forms, including rhythmic splicing in otherwise arrhythmic transcripts [55•]. Many instances of rhythmic ncRNAs are also apparent in Arabidopsis, including microRNAs (miRNAs), trans-acting short interfering RNAs, small nucleolar RNA, and RNAs derived from over 1000 intergenic regions in the genome [55•]. These novel examples of clock regulation for ncRNAs extend the purview of the circadian oscillator beyond protein coding transcripts. In support of this idea, two circadian regulated miRNAs, *miR160B* and *miR167D*, target members of the ARF transcription factor family that regulate expression of auxin-responsive genes [56]. *miR160B* targets *ARF10*, *ARF16*, and *ARF17*, which are though to be involved in growth processes surrounding germination [57]. *miR167D* targets *ARF6* and *ARF8*, which are involved in male and female reproductive development [58]. Given that ncRNAs act in key developmental processes in plants and animals [59,60], these examples from Arabidopsis may represent the dawning of a new, exciting area of research into the intersection of circadian regulation and plant development.

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